Meeting report

Novel insights into proteomic technologies and their clinical perspective

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Abstract

A report on the Proteomic Forum 2009 conference, Berlin, Germany, 29 March to 2 April, 2009.

Continuing a highly successful series of meetings originally established by Angelika Görg in Munich, scientists gathered for the first Proteomic Forum in Berlin. The five-day meeting organized by the German Society of Proteome Research included plenary lectures, poster sessions and workshops focusing on new developments in the field of proteome science. With about 500 participants, 60 speakers and 100 company representatives, the meeting provided a broad overview of the field. While virtually all areas of proteomics were presented, a clear trend towards mass spectrometry-based workflows was evident. Featured topics discussed in this report include proteomic techniques, biomarker discovery, proteomics of molecular machines and cell signaling.

Proteomic techniques

The increased mass accuracy, dynamic range, speed and sensitivity of mass spectrometers is currently the driving force for the rapid expansion in the number and quality of proteomic datasets. As the depth of proteome coverage increases, obtaining quantitative data becomes increasingly more important. Hence, quantitative proteomics was a common theme of the meeting, with methods ranging from simple spectral counting to stable isotope labeling-based approaches, such as stable isotope labeling with amino acids in cell culture (SILAC). John Yates III (The Scripps Research Institute, CA, USA) presented data on the use of spectral counting for label-free quantification to investigate the interactome of the cystic fibrosis transmembrane conductance regulator (CFTR). Importantly, quantification facilitates differentiation between specific interaction partners and non-specific contaminants, as well as assessment of the effect of drugs on the interactome. Yates also presented ^{15}N labeling of rats as another approach for *in vivo* quantification. Christoph Turk (Max Planck Institute of Psychiatry, Munich, Germany) added a word of caution to this, as he found that ^{15}N labeling of mice significantly affects animal behavior.

A new advancement for targeted proteomics was presented by Ruedi Aebersold (Swiss Federal Institute of Technology (ETH) Zurich, Switzerland). In an effort to expand the use of multiple reaction monitoring (MRM) for proteomic purposes, the group used data from large-scale identifications and arrays of synthesized peptides to generate a library of key MRM transitions for all yeast proteins. These transitions can be used to identify and quantify, by MRM techniques, proteins expressed at only 50 copies per cell. A number of groups presented new bioinformatics approaches for the prediction of MRM transitions based solely on sequence information.

Several other approaches were presented that extend the analytic abilities of mass spectrometry-based workflows. For example, Kris Gevaert and Joel Vandekerckhove (Ghent University, Belgium) reported on the use of combined fractional diagonal chromatography (COFRADIC) for the identification of protease target sites. Albert Heck (Nether-

lands Proteomics Centre, Utrecht University, The Netherlands) demonstrated that using the protease Lys-N in combination with electron transfer dissociation (ETD) greatly facilitates *de novo* sequencing of peptides and identification of post-translational modifications.

Biomarkers

Identifying reliable markers indicative of a disease state from body fluids has been reported to be a very daunting task. As proteomic technologies have improved considerably over recent years, the quest for biomarkers is becoming popular again. A particularly promising source for potential biomarkers is the secretome of diseased cells. Many cells also release membranous vesicles (exosomes) that may provide diagnostic information. Richard Simpson (Ludwig Institute for Cancer Research, Melbourne, Australia) used secretome data to identify proteins that are possibly indicative of colon cancer. Thomas Conrads (University of Pittsburgh, PA, USA) described a similar approach for the identification of new biomarkers for prostate cancer. Richard Caprioli (Vanderbilt University, TN, USA) reported on the use of imaging matrix-assisted laser desorption ionization (MALDI) to analyze biopsies obtained from cancer patients. The spatial resolution of his data reveals that tumor markers sometimes extend beyond the tumor margin into seemingly healthy adjacent tissue. This indicates that surgical margins may need to be reconsidered in some cases.

Molecular machines and protein interactions

The analysis of complex molecular machines was the focus of two sessions at the meeting. Several studies presented employed a proteomic description of a complex, and deduced substructures for analysis with more traditional methods. Reinhard Lührmann (Max Planck Institute for Biophysical Chemistry, Goettingen, Germany) presented work on yeast and human spliceosomes revealing a high-resolution map of the active complexes. The proteomic data nicely defined subcomplexes, such as the NineTeen subcomplex of the spliceosome. In collaboration with Henning Urlaub, the group used a combination of mass spectrometry and UV crosslinking to identify interacting complexes within the spliceosome. Another long-standing goal is to use protein interaction data to understand disease phenotypes. Marius Ueffing (Helmholtz Zentrum Munich, Germany) used tandem affinity purification to define the interactome of lebercilin, a protein genetically linked to a severe form of blindness. Interestingly, the identified interaction partners linked the disease to vesicular transport, highlighting new candidate disease genes.

Kinase signaling

Large-scale identification of phosphorylation sites is another important dimension in proteomics. Roland Annan (SmithKline Beecham Pharmaceuticals, PA, USA) and Ole Jensen (University of Southern Denmark, Odense, Denmark) presented a number of new techniques used for the identification of new phosphorylation sites in complex mixtures. The use of affinity matrices, special precipitation techniques and novel chromatographic strategies, such as hydrophilic interaction liquid chromatography (HILIC), proved to be particularly valuable. Combined with SILAC-based quantification, these methods allowed for a systematic comparison of the phosphoproteome of drug-resistant and drug-sensitive breast cancer cells. These data may facilitate a rational selection of chemotherapeutic agents to treat cancer. As Boris Macek (Proteome Center Tübingen, Germany) pointed out, large-scale SILAC-based quantification of phosphorylation is not limited to eukaryotes, but also reveals that phosphorylation is dynamically regulated in prokaryotes.

One problem in large-scale mapping of phosphorylation sites is that this method alone cannot reveal direct kinase-substrate relationships. Two groups showed that so-called analog-sensitive kinases can be employed to alleviate this problem. Hendrik Daub (Max Planck Institute of Biochemistry, Martinsried, Germany) used a modified form of Plk1 designed to respond to a specific inhibitory compound. Cells expressing either this analog-sensitive kinase or wild-type Plk1 were treated with the inhibitor. SILAC-based quantification of phosphopeptides from both conditions allowed identification of likely direct Plk1 substrates.

Concluding remarks

The meeting provided an interesting snapshot of current approaches and questions in proteomic science. As a forum in the best sense of the word, the Proteomic Forum provided a public space for many fruitful discussions. Given the current speed at which the field is evolving, reviving the meeting series was a logical consequence and proved to be a great success. The improved analytic abilities of modern instruments are beginning to transform basic research and will have an impact on clinical proteomics. Ultimately, this may bring mass spectrometers to the doctor's office - the dream of early pioneers like John Fenn.

Abbreviations

CFTR, cystic fibrosis transmembrane conductance regulator; COFRADIC, combined fractional diagonal chromatography; ETD, electron transfer dissociation; HILIC, hydrophilic interaction liquid chromatography; MALDI, matrix-assisted laser desorption ionization; MRM, multiple reaction monitoring; SILAC, stable isotope labeling with amino acids in cell culture.

Competing interests

The authors declare that they have no competing financial interests.